Direct Detection And Quantification of HIV

- Early Detection
  Does not depend on development of antibodies
- Direct
  Detects HIV DNA directly
- Sensitive
  As few as 10 copies of HIV DNA can be detected
- Specific
  Two hybridization events are required for a positive signal
- Developed with Enzo's proprietary technology

Enzo Clinical Labs

A test for the direct detection and quantification of the HIV virus is available from Enzo Clinical Labs. This test was developed by Enzo Diagnostics, using its proprietary microplate hybridization technology.

HIV DNA detection offers a distinct advantage over currently used serological assays. The Enzo test identifies the presence of HIV DNA sequences rather than the presence of HIV antibodies that may not appear until months after the initial infection. This test offers the potential to identify DNA in instances where HIV positivity cannot be identified adequately in samples from HIV positive individuals who have not seroconverted.

Enzo's assay has several features that make it unique. The use of probe pairs increases the specificity of the assay. Two independent hybridization events are required to generate a signal. This assay has been found to be insensitive to the presence of cellular components other than the target DNA.

With amplified samples, the ultimate sensitivity of an assay is dependent upon the amplification efficiency. Using the microplate assay, as few as 10 copies of HIV DNA could be detected.

The test also lends itself to quantification of viral load. Such information is useful for measuring the effect of drug treatments on virus concentration, virus concentration during the course of infection, virus concentration in animal model studies and numerous other applications where virus quantification is a critical parameter.

Summary of Collaborative Studies

The performance of Enzo's HIV Microplate A
collaboration with the Division of HIV/AIDS a
The following is a summary of some of thes

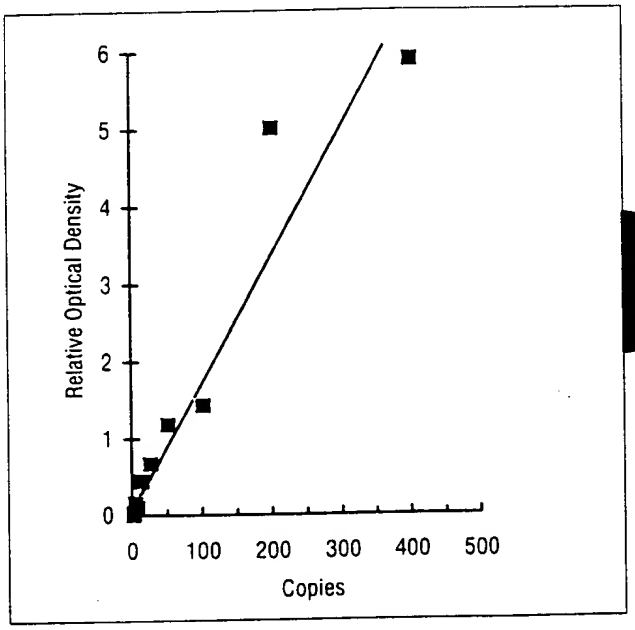
Analytic Sensitivity

In an initial set of experiments in which a series of DNA samples containing known amounts of HIV DNA were amplified and then assayed using Enzo's Microplate Assay, fewer than 10 copies of HIV proviral DNA could be detected (Fig. 1). These data suggested that application of this technology to the problem of HIV detection in clinical samples might allow the identification of HIV positive individuals who have not yet seroconverted.

Figure 1

Detection of HIV Sequences in Amplified Samples

Samples represented 1 μg of human DNA that was amplified for 35 rounds in the presence of the indicated number of copies of cloned HIV DNA.



ssay was evaluated in studies undertaken in
t the Centers for Disease Control in Atlanta.
studies.

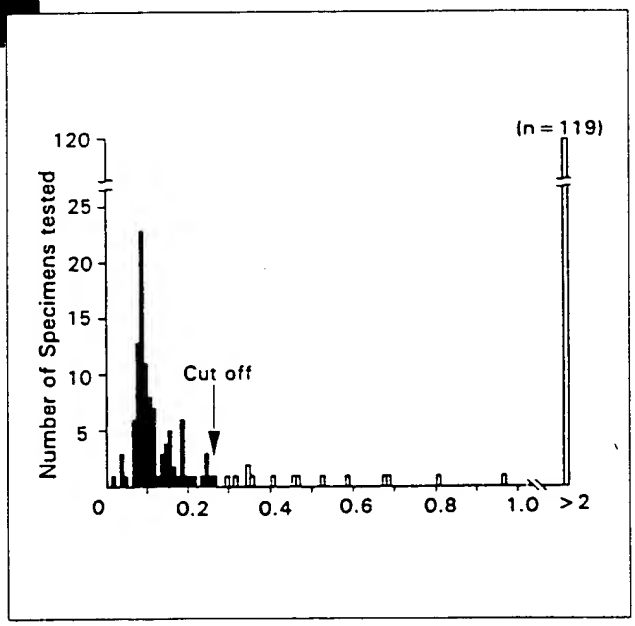
Clear Cut-Off Point

In any clinical assay the negative cut-off value, or the point at which a sample can be determined to be positive is extremely important. To investigate this, 104 seronegative and 142 seropositive individuals were examined using the Microplate Assay and the results are shown in Fig. 2 below.

Figure 2

Negative Cut-Off Value

Samples from positive and negative specimens plotted to demonstrate the clear negative cut off value obtained with the assay.



All DNA samples from negative individuals tested negative. The average ± 3SD (99% confidence level) of the 104 negative specimens was 0.113 ± 0.156. The mean + 3SD (0.269) was used as the cut-off. Of the 142 positive specimens 84% gave readings >2. Thus as can be seen in figure 2, the microplate assay can provide a clear discrimination between positive and negative individuals.

Low Levels of HIV Detected

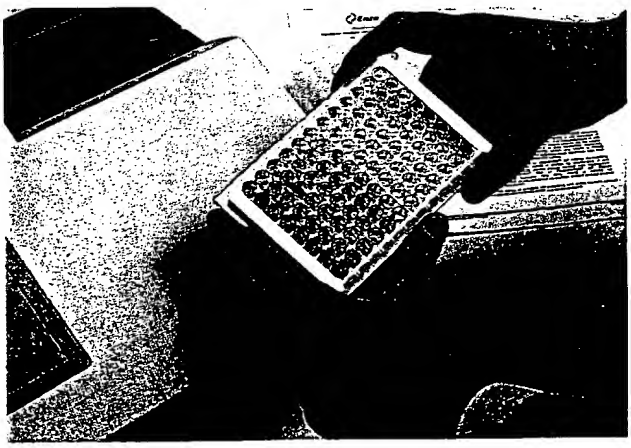
When enzymatically amplified HIV DNA, from either the *gag* or *env* regions was the target, the assay was able to detect HIV DNA in all (36/36) samples from seropositive individuals. These results were in agreement with a radioactive "gel assay" performed in parallel on the same samples. Furthermore, the assay detected HIV DNA after amplification in 6/6 samples taken from individuals who were seronegative at the time of sampling, but who subsequently seroconverted. These results confirm the applicability of the assay for the early detection of low levels of HIV.

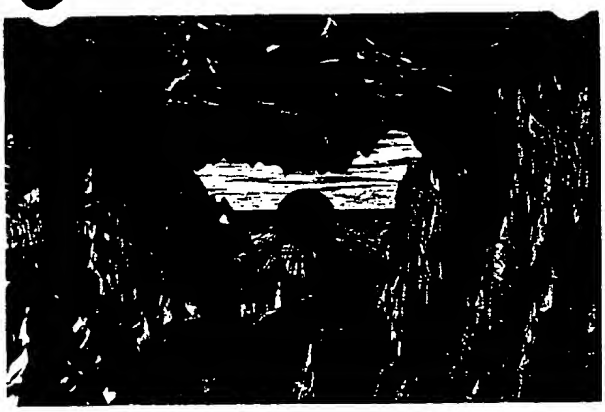
References

Cook, A.F. *et al.* (1988) Synthesis and hybridization of a series of biotinylated oligonucleotides. Nuc. Acid Res. 16:4077-4095.

Rapier, J.M. *et al.* (1993) Nonradioactive, colorimetric microplate hybridization assay for detecting amplified human immunodeficiency virus DNA. Clin. Chem. 39:244-247.

Lee, L.S. *et al.* (1990) A nonisotopic hybrid capture assay for HIV nucleic acid sequences. Abstract US-CAP Academy of Pathology, March 1990.





Test Information:
Enzo HIV Microplate Assay

Number: 1410 - Qualitative
Number: 1411 - Quantitative

Specimen Requirement

1 Lavender top tube (EDTA)

Reference

Negative - no detectable amount of HIV-1 DNA found

Method

Enzo HIV Microplate Assay System

Comments

This test is for research use only, and is not to be used as a diagnostic procedure without confirmation of the diagnosis by other medically established means.

Enzo Clinical Labs, Inc.
60 Executive Boulevard
Farmingdale, NY 11735
800 - 522 - 5052